REMARKS

Reconsideration and allowance of the present application are respectfully requested.

Claims 1-8 and 11-12 are in this case. Claims 1, 2 and 6 have been amended. Claims 9-10 have been cancelled. Claims 11-12 have been added.

Before turning to the rejections stated in the Office Action, the applicants provide the following preliminary comments which are believed to be helpful in further understanding the patentability of the presently claimed invention.

The DNA construct of the present invention is useful for repeatedly carrying out a transformation of the gene of interest and excising a selective marker gene.

As described in the present specification (see lines 8-16 of page 3),

> "A known FRT sequence is a 34-bp sequence consisting of a spacer sequence of 8 bp and inverted repeats of 13 bp....However, this 34-bp FRT sequence is not suitable for commercial use because site-specific recombination using this sequence leaves recognition sequences of the recombinase on chromosomes after recombination so that undesired recombination may be induced." (page 3, lines 8-16)

When the first transformation is carried out using the construct having the FRT sequences, single FRT sequence resides in the yeast chromosome after the recombination by the FLP product and the excision of a selective marker gene (see Figure 6). If both of the FRT sequences used in the first transformation are the same as a wild-type sequence (SEQ ID NO: 1 in the present invention), the single FRT sequence that resides in the yeast chromosome is a "wild-type" sequence (SEQ ID NO: 1).

Subsequently, if the second transformation is also carried out using the construct having the FRT sequences, the FRT sequences used in the second transformation may randomly recombine with the single wild-type FRT sequence residing in the chromosome as debris of the previous transformation. Therefore, "undesired recombination may be induced" and the "FRT sequence is not suitable for commercial use." This is the problem that was to be solved as of the priority date of the present application.

The main feature of the construct of the present invention is that one FRT sequence which exists at the 5' side of the selective marker gene has at least 1 but not more than 4 nucleotides deleted from the 5' end of the inverted repeat (1) thereof and that the other FRT sequence which exists at the 3' side of the selective marker gene has at least 1 but not more than 5 nucleotides deleted from the 3' end of the inverted repeat (2) (see presently amended claim 1). Using this construct of the present invention, the single FRT sequence residing in the yeast chromosome has at least 1 but not more than 4 nucleotides deleted at the 5' end, and has at least 1 but not more than 5 nucleotides deleted at the 3' end thereof after the transformation (see Figure 7). The examples of such FRT sequences resided in the yeast chromosome having at least 1 nucleotide deletions at both ends are FRT2W (SEQ ID NO: 4) and FRT3W (SEQ ID NO: 7) (see Figure 3).

Upon subsequent transformation, it is difficult for such FRT sequence having nucleotide deletion at both ends to recombine with the FRT sequences derived from the construct of the invention. Undesired recombination is not likely to be induced and, therefore, the construct of the invention is suitable for commercial use.

Turning now to the rejections stated in the Office Action, the applicants respectfully traverse the rejection of claims 1-6 and 9-10 under the judicially created doctrine of obviousness-type double patenting over claims 1-7 of U.S. patent no. 5,965,444 (US '444) in view of Kawahata et al. Claims 9-10 have been cancelled.

US '444 claims a DNA construct using the Zygosaccharomyces rouxii-derived site-specific recombination system R/RS (claim 1) and a method for transforming yeast using the DNA construct of claim 1 (claim 4). Specifically, claim 1 of US '444 describes that "A DNA construct comprising an **R gene** positioned under the control of an inducible promoter, and an expressible selective maker wherein the **R gene** and the selective marker are flanked by a pair of **R sensitive sequences** oriented in the same direction so as to form a removing unit...."

In contrast, the presently claimed invention recited a DNA construct using the characteristic FRT/Flp recombination system and GIN sequence (claims 1 and 11-12) and a method for transforming yeast using the DNA constructs of claims 1-3 (claim 2).

The presently claimed invention differs from the invention of US '444 by at least the following features:

(a) Recombination system of the present invention is **FRT/Flp** system, while that of the invention of US '444 is **R gene/R sensitive sequences** system;

(b) Growth inhibitory sequence (GIN) is comprised in the construct of the present invention, while such a sequence is not included in the construct of the invention of US '444;

(c) In the presently claimed invention, even the construct containing FRT3W sequence, which has deletions at both ends, exhibits 100 % of site-specific recombination (see, page 30, lines 18-20 of the present specification); while, in the invention of US '444, the construct containing RE4W sequence, which has deletions at both ends, only exhibits 37 % of site-specific recombination (see, Table 1 in '444).

The above characteristic (c) of the presently claimed invention is generated by the unique structural feature of the construct of the invention as described in the above preliminary comments.

Furthermore, the construct of the presently claimed invention is useful for repeatedly carrying out a transformation of the gene of interest, and for excising a selective marker gene, as described in the preliminary comments, above.

The teachings of Kawahata et al. do not remedy the deficiencies of US '444.

Accordingly, the applicants submit that since the construct of the present invention definitely and significantly differs from that of the claims of US '444 in view of its structure and exhibits more recombination frequency than that of US '444, the presently claimed invention is fully allowable under Double Patenting in view of the claims of US '444 in view of Kawahata. Withdrawal of this rejection is respectfully requested.

The applicants respectfully traverse the rejection of claims 1-8 and 9-10 under 35 USC 112, first paragraph, because the Examiner believes that these

claims contain subject matter inadequately described in the specification. Claims 9-10 have been cancelled.

More particularly, the Examiner appears to believe that the written description requirement of Section 112, first paragraph has not been satisfied because those ordinarily skilled in the art could not have carried out the DNA construct having a sequence "substantially identical to" the native FRT sequence.

Please note that previously considered claim 1 has been divided by amendment and addition of claims herein, into amended claim 1 and new claims 11-12. The FRT sequences used are restricted to those having nucleotides deletion based on the description of the Examples and the drawings.

As described in the preliminary comments above, a main feature of the presently claimed invention is that one FRT sequence which exists at the 5' side of the selective marker gene has at least 1 but not more than 4 nucleotides deleted from the 5' end of the inverted repeat (1) thereof and that the other FRT sequence which exists at the 3' side of the selective marker gene has at least 1 but not more than 5 nucleotides deleted from the 3' end of the inverted repeat (2) such that both ends of the single FRT sequence resides in the yeast chromosome after the transformation were truncated by at least 1 nucleotide.

This feature is achieved for the first time by the presently claimed invention, as described in the present specification and the drawings. Specifically, the experiment of the transformation and the excision of a selective gene were actually carried out using the construct of the presently claimed invention (see, Examples 2 and 3).

Additionally, the invention of claim 12 is supported by the description in the specification that

> "A pair of FRT sequences used in a DNA construct prepared in the examples below and sequences reconstructed by recombination from these sequences are illustrated in Fig. 3. Among them, combinations FRT2/FRT102 and FRT3/FRT103 are preferred pairs of FRT sequences for use in DNA constructs of the present invention" (lines 4-9, page 12 of the present specification).

Example 1 of the present specification describes that a recombination event actually occurred when yeast was transformed by each DNA construct having deletion mutations such as the combinations FRT2/FRT102 and FRT3/FRT103. Examples 2 and 3 describe that a selective marker gene and a drug resistance selective gene which were originally contained in the plasmid pPUGINFRT3-103 as produced in Example 1, were effectively removed from the yeast chromosome which is transformed using the plasmid pPUGINFRT3-103.

As described in the present specification, the recombination frequency of the plasmid containing the combination FRT2/FRT102 or FRT3/FRT103 is lower than that of the plasmid containing the combination FRT1/FRT101 (wild-type) (see, Table 1 in page 26). However, since the recombined yeast cells were effectively selected by a selective marker gene and, then, the selective marker gene was efficiently excised and removed from the yeast chromosome (Examples 2 and 3) and is useful for transformation repeatedly, the plasmid containing the combination FRT2/FRT102 or FRT3/FRT103 exhibited an even higher efficiency.

Accordingly, the applicants submit that presently amended claim 1 and new claims 11-12 are fully supported by the specific embodiment of the present

invention as described in the specification and the drawings and, therefore, the invention of claims 1 and 11-12 is sufficiently described in the specification and the drawings in full, clear, concise and exact terms that fully satisfy Section 112, first paragraph. Withdrawal of this rejection is respectfully requested.

The applicants respectfully traverse the rejection of claim 8 under 35 USC 102(b) in view of Omura. This reference does not anticipate the presently claimed invention or make it obvious.

However, please note that claim 8 depends upon claim 7 which together with its further base claims have been shown above to be fully allowable. Thus, since claim 8 depends upon allowable base claims, then claim 8 should be found allowable.

The applicants respectfully traverse the rejection of claim 6 under 35 USC 103(a) over Ashikari in view of Kawahata. These references do not make the presently claimed invention to be obvious.

The presently claimed invention is based on the technical concept which uses FRT sequences/Flp system for the recombination of yeast and for the excision of selective marker genes. Further, the present invention demonstrates a significant advantageous effect as described in the preliminary comments, above.

Ashikari describes an invention using the Zygosaccharomyces rouxii-derived site-specific recombination system R/RS in Saccharomyces cerevisiae. The reference describes problems in the invention of Ashikari that, since the introduction of a foreign gene for recombinase (R gene) is involved in the invention, the excision efficiency of the selective marker gene varies with the strain of yeast to be transformed.

Kawahata describes excision of selective marker genes using growth inhibition sequence (GIN sequence). The method of Kawahata successfully removes the selective marker gene at an efficiency of 96% or more. However, there are problems in the invention of Kawahata wherein this method is not suitable for commercial use because the selective marker gene is excised by homologous recombination to leave an unnecessarily long sequence as an excision mark of the selective gene on the chromosome.

Importantly, there is no suggestion or disclosure in Ashikari or Kawahata for using the DNA construct as recited in amended claim 1 and new claims 11-12 which have characteristic structures in comparison to the construct of the prior art and the FRT/Flp system of the present invention to recombine the yeast chromosome. Neither Ashikari or Kawahata provides a motivation to realize the concept of FRT/Flp system. Even if those of ordinary skill in the art combined the disclosure of these citations, they would not be able to realize the invention of present claim 6.

The feature of the yeast of the invention, which is generated by the method of claims 3-5, is that the yeast, after the transformation and the recombination, contains the single FRT sequence residing in the yeast chromosome having at least 1 but not more than 4 nucleotides deletion at the 5' end, and at least 1 but not more than 5 nucleotides deletion at the 3' end thereof after the transformation. Such yeast can be used as a parent strain repeatedly for the further transformation as described in Section (1) above in detail.

These are significant effects of the yeast of the presently claimed invention, which can not be expected from the teachings of Ashikari in view of Kawahata.

The applicants submit that the presently claimed invention is fully allowable under Section 103(a) in view of the teachings of Ashikari combined with the teachings of Kawahata.

In view of the above, it is believed that this application is in condition for allowance and a Notice to that effect is respectfully requested.

Respectfully submitted,

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